Citation:

Harman NL, Leeds AR, Griffin BA. Increased dietary cholesterol does not increase plasma low density lipoprotein when accompanied by an energy-restricted diet and weight loss. *Eur J Nutr.* 2008 Sep;47(6):287-93. Epub 2008 Aug 26. Erratum in: *Eur J Nutr.* 2008 Oct;47(7):408.

PubMed ID: 18726564

Study Design:

Randomized Controlled Trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

- It has been observed that the energy deficit that produces weight loss and/or the weight loss itself may counter affect the cholesterol-raising effects of increased dietary cholesterol
- The study tested that hypothesis and compared the combined effects of two energy-restricted diets, with and without added dietary cholesterol (2 eggs/day), on weight loss, plasma lipids and lipoproteins

Inclusion Criteria:

- Healthy male and female volunteers
- Aged 18-55 years old
- Had to be free of any medical condition or medication that would adversely affect lipid metabolism or prevent them from adhering to the study protocol
- Willing to eat eggs
- Willing to refrain from consuming dietary supplements or cholesterol lowering 'functional' foods, chiefly products containing plant sterols or enriched with soy protein 2 weeks prior to baseline measurement and for the duration of the intervention

Exclusion Criteria:

- BMI>35
- Plasma total cholesterol >6.5 mmol/l
- Plasma triglyceride > 3mmol/l
- Being on an existing diet or having lost >3kg in weight in the preceding 2 months

Description of Study Protocol:

Recruitment

Participants were recruited through advertisement in local newspapers.

Design: Randomized, controlled, parallel trial

Blinding used (if applicable): none mentioned

Intervention (if applicable)

- Open design, subjects randomly assigned to one of two parallel dietary interventions:
  - energy restricted diet which included two eggs per day for 12 weeks
  - same energy restricted diet without eggs ('control') for 12 weeks
- Diet aimed to reduce energy intake by between 500-1000 kcal per day, by limiting portion size and the consumption of both dietary fat and carbohydrates
- Egg fed group was provided with medium size eggs from a single supplier throughout the study
- All subjects completed an initial 7 day food diary to establish their habitual intake, after which, each subject received dietetic counseling and individualized diet plan to follow for 12 weeks
- Weight loss was monitored during the study by regular meetings with a dietitian
- Another 7 day food diary was completed after 6 weeks of dietary intervention to check on dietary compliance
- each subject was provided with a low fat (fat <5% energy) supermarket 'ready meal' as a pre-visit meal to consume by 8 pm the night before their baseline, 6- and 12-week visits to standardize both short and longer term variation in dietary intake within and between subjects

Statistical Analysis

- Tested for two possible outcomes: that the cholesterol-enriched, energy restricted diet could either be 'equivalent' or 'superior' in its effects on plasma LDL cholesterol to that of the energy restricted diet alone
- All biochemical data, body weight and percentage body fat were logarithmically transformed for statistical analysis - results expressed as mean±Standard Deviation (SD)
- Difference between egg-fed and non-egg fed groups were examined by ANOVA with time as a repeated measure using a General Linear Model (GLM) with 'treatment' (egg-fed vs non-egg fed) and 'time' (0, 6, 12 weeks) as factors
- Within and between group differences between baseline and 12 weeks were determined by one and two-sample students t-test, respectively, with a Bonferroni correction for multiple comparisons

Data Collection Summary:

Timing of Measurements

Subject appointments were between 8 am and 1 am, after a 12-hour fast

Dependent Variables

- Anthropometrics and body composition: height, weight, waist circumference, blood pressure and percent body fat measured by bioelectrical impedance
- Total Plasma Cholesterol and Triglycerides - measured by commercially available

enzymatic assays
- LDL-cholesterol measured directly using 'LDL Direct' kit with the use of a SpACE autoanalyzer. It was also calculated using Friedewald formula
- LDL subclass distribution - measured by iodixanol density gradient centrifugation using LDL density as a surrogate of LDL particle size
- HDL-cholesterol measured directly using 'HDL Direct' kit with the use of a SpACE autoanalyzer.
- Plasma glucose-measured by commercially available colorimetric
- Insulin was measured with ELISA assay

Independent Variables

- Energy restricted diet with one group instructed to consume 2 eggs per day and the other to exclude eggs, for 12 weeks

Control Variables

Description of Actual Data Sample:

Initial N: 67 initially recruited. 53 met study entry criteria and were randomly assigned to either energy restricted diet with no eggs (control, n=26) or the energy-restricted diet plus two eggs per day (n=27)

Attrition (final N):

Eight subjects withdrew for personal reasons or illness (n=5), pregnancy (n=1) or failure to adhere to the study protocol (n=2).

Final N=45

- egg fed group n=24
- non-egg fed group n=21

Age:

- egg fed group: mean 44.9 (8.4)
- non egg fed group: mean 43.0 (10.5)

Ethnicity:not described

Other relevant demographics:

Anthropometrics

No significant difference between dietary groups in age, weight or concentration of plasma lipids and lipoproteins at baseline.

	Egg Fed (n=24) **mean(SD)**	**Non-Egg Fed (n=21)** **mean(SD)**
Sex (M/F)	8/17	6/15

Weight (kg)	84.2 (15.9)	83.2(15.7)
Body Mass Index (kg/m^2)	30.1(3.7)	29.0(4.2)
Waist Circumference (cm)	97.5(13.6)	90.1(12.1)
Blood Pressure (mm Hg)		
systolic	120(13)	126(20)
diastolic	79(7)	78(10)
Plasma glucose (mmol/l)	5.78(0.65)	5.90(0.53)
Plasma Cholesterol (mmol/l)	5.21(0.79)	5.30(1.16)

Location:Guilford (Surrey), GU2 7XH, UK

Summary of Results:

Key Findings:

- Energy intake fell by 25 and 29% in the egg-fed and non-egg-fed groups, resulting in a moderate weight loss of 3.4 kg ($P < 0.05$) and 4.4 kg ($P < 0.05$), respectively.
- The daily intake of dietary cholesterol increased significantly in the egg-fed group from 278 to 582 mg after 6 weeks.
- The concentration of plasma LDL cholesterol decreased in the non-egg-fed groups after 6 weeks ($P < 0.01$) and in the egg-fed and non-egg-fed at 12 weeks relative to baseline.
- There were not other significant changes in plasma lipoproteins or LDL particle size.

Body Weight, Plasma Lipids, Lipoproteins and Apoproteins at Baseline and after 6 and 12 weeks of Dietary Intervention

	Baseline (mean±SD) Egg fed vs Non-egg fed	6 weeks (mean±SD) Egg fed vs Non-egg fed	12 weeks (mean±SD) Egg fed vs Non-egg fed	Change (%) Egg fed vs Non-egg fed
Weight (kg)	81.0±16.6 vs 80.7±15.3	78.6±16.0^ vs 77.3±14.8^	77.6±16.0^ vs 76.4±14.7^	n/a
Body fat (%)	34.9±7.9 vs 33.6±7.4	34.3±7.2 vs 32.5±8.4	33.3±7.9 vs 31.2±8.9	n/a
Lipids (mmol/l)				
Total Cholesterol	5.34±1.14 vs 5.20±0.99	5.29±1.15 vs 4.63±0.94	4.99±1.24 vs 4.98±0.91	n/a

LDL Cholesterol	3.02±0.76 vs 2.95±0.66	2.97±0.78 vs 2.72±0.49*	2.85±0.78 vs 2.85±0.51	n/a
HDL Cholesterol	1.12±0.28 vs 1.08±0.36	1.08±0.31 vs 0.92±0.35	0.97±0.30 vs 1.10±0.32	n/a
Triglyceride	1.20±0.56 1.28±0.59	1.23±0.62 vs 1.17±0.48	1.11±0.44 vs 1.21±0.72	n/a
LDL peak density (g/l)	1.0243 vs 1.0252	1.0243 vs 1.0250	1.0237 vs 1.0251	n/a
%sdLDL (median)	14.8±10.1 vs 18.4±16.2	15.7±12.4 vs 18.5±17.2	13.5±10.7 vs 20.1±13.5	n/a
Apoproteins (g/l)				
Apoprotein A-I	1.28±0.27 vs 1.19±0.22	1.21±0.27 vs 1.09±0.20	1.12±0.28 vs 1.18±0.20	n/a
Apoprotein B	1.03±0.25 vs 1.04±0.19	1.17±0.25 vs 1.25±0.18	1.18±0.20 vs 1.14±0.23	n/a

*Treatment effect (P<0.01) by General Linear Model, with a post hoc Turkey pairwise comparison

^ within group differences (P<0.05) after 6 and 12 weeks versus baseline, by student's paired t test with Bonferroni correction for multiple comparisons

Author Conclusion:

In conclusion, this study demonstrates that increasing dietary cholesterol by consuming two eggs a day, produces no increase in plasma LDL when accompanied by energy restriction and moderate weight loss. The latter may also counteract any cholesterol-raising potential of increased dietary cholesterol, at least in the short term. These findings support the view that cholesterol-rich foods should not be excluded from an energy-restricted diet on account of producing an unfavorable effect on blood cholesterol.

Reviewer Comments:

Supported by the British Egg Industry Council. Some of the limitations discussed are as follows:

- *The reduced amount of weight lost between 6 and 12 weeks strongly suggests that the energy deficit was not maintained over this period*
- *Non-egg consumers lost marginally more weight than the egg-fed group.*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions

1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions

1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	No
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	No
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A

3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	Yes
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	No
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes

6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	Yes
6.6.	Were extra or unplanned treatments described?	Yes
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	Yes
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	N/A
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	Yes
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes

9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	???
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	No